XVIII. THE RATE OF FERMENTATION BY GROWING YEAST CELLS.

By ARTHUR SLATOR.

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If a sugar solution which also contains the necessary food for yeast growth is seeded with a small quantity of yeast, the yeast grows and the sugar is fermented to alcohol and carbon dioxide. If the seeding is small and all necessary food for yeast growth is in excess the growth during the earlier stages of the reaction is unrestricted and follows the logarithmic law of increase, that is the rate of increase is always proportional to the quantity present.

At a later period retarding influences come into action, the yeast multiplication becomes restricted and during the final stages of the fermentation ceases entirely.

In this communication an account is given of some measurements made during the earlier part of the reaction where the yeast growth is unrestricted.

If certain simple assumptions regarding fermentation are made, it can be shown that during this period not only does yeast growth, but also the fermentation caused by the yeast, follow the logarithmic law.

If the medium is seeded with N cells per c.c. then the rate of growth at any time t is proportional to the number of cells present $N+n$, where n is the increase during the time t, that is,

$$\frac{dN}{dt} = K(N+n) \quad \text{.....(1).}$$

where K is the constant of growth.

On integration this becomes

$$K = \frac{1}{t} ln \frac{N+n}{N} \quad \text{.....(2),}$$

or

$$n = N(e^{Kt} - 1) \quad \text{.....(2 a).}$$

If F represents the number of grams of sugar fermented per unit of time by each yeast cell and s the total amount fermented in time t, then s is determined by the equation

$$s = \int_0^t (N+n) F \, dt.$$

Substituting from equation 2 a we have

$$s = \int_0^t NF e^{Kt}\, dt$$

which on integration becomes

$$s = \frac{NF}{K}(e^{Kt} - 1) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3),$$

or from equation 2 a

$$s = \frac{nF}{K} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4).$$

If S is the amount of sugar fermented when the yeast grows from a very small seeding to N, then from equation 4

$$S = \frac{NF}{K} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5).$$

Equation 3 therefore becomes

$$s = S(e^{Kt} - 1) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

or

$$K = \frac{1}{t} ln \frac{S+s}{S} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6a).$$

It is evident from this equation that the time-fermentation curve during this period is logarithmic and that the constant of the curve is the constant of growth.

The validity of these equations has been tested by measuring the rate of growth of a pure culture of a Burton yeast in lightly hopped wort of specific gravity 1·040. It was found that K the constant of unrestricted growth of this culture could be determined by methods of yeast counting and also by measuring the rate of fermentation of the growing yeast cells.

Four different methods of estimating K have been worked out and when tested with this growth of yeast were found to give almost identical results.

In all these experiments the seeding was made with actively growing yeast cells so that any initial retardation in the growth which would take place if older yeast cells were used is eliminated.

Method 1.

The medium is seeded with a known number of yeast cells under sterile conditions. Tubes containing the inoculated solution are kept slowly agitated in a thermostat. At certain intervals of time the tubes are taken out and the number of yeast cells counted. K is calculated according to equation 2 which can be put in the form

$$0{\cdot}434\, K = \frac{1}{t} \log \frac{N+n}{N}.$$

TABLE I.

Temp. 20°.
t = time in hours.
$N+n$ = number of yeast cells per c.c. at time t.

t	$N+n$	$0{\cdot}434\,K$
0	90,100	—
17·3	4,660,000	0·100
0	255,000	—
7·0	1,390,000	0·105
9·0	2,200,000	0·104
0	1,360	—
35	3,550,000	0·098
	Average	0·102

$T = 2{\cdot}95$ hrs.

In the above table T is the time for the yeast to increase to twice the original amount and is determined by the equation

$$T = \frac{\log 2}{0{\cdot}434\,K} = \frac{0{\cdot}301}{0{\cdot}434\,K}.$$

METHOD 2.

Measurements of the rate of fermentation are carried out in the manner described in previous publications. [Slator, 1906, 1908.]

The sterile medium (75 c.c.) is seeded with a small seeding of yeast in a tube of capacity about 100 c.c. The tube is connected with a manometer to measure the rate of production of carbon dioxide. With a suitable seeding appreciable fermentation starts in about 15 hours, the reaction proceeds faster as the time goes on and measurements are continually taken on the manometer at suitable intervals.

The readings on the scale can be used directly to test equation 6 a

$$0{\cdot}434\,K = \frac{1}{t}\log\frac{S+s}{S}.$$

TABLE II.

Temp. = 20°.
t = time in hours from first reading S.
$S+s$ = manometer reading in cm., at time t taking the infinitely early reading as zero.

t	$S+s$	$0{\cdot}434\,K$
−15	(0·3)*	—
0	9·7 (S)	—
0·5	10·9	0·101
0·83	11·85	0·105
1·25	12·9	0·099
1·58	13·9	0·099
1·92	15·05	0·099
	Average =	0·101

A second experiment gave $0{\cdot}434\,K = 0{\cdot}100$. $T = 3{\cdot}0$ hrs.

* NOTE. 15 hrs. before the first reading is too short a time to give exactly the infinitely early reading and a small correction has to be made to obtain this value. As T is approximately 3 hrs. this correction is easily shown to be 1/32 of S, that is in this case 0·3 cm.

Method 3.

The constant of growth can be estimated by means of equation 5

$$K = \frac{FN}{S}.$$

The yeast crop and the amount of fermentation are measured after a convenient amount of fermentation has taken place, the initial seeding of yeast being small. F is determined by a separate experiment. The method is not of much practical value as both N and F have to be determined whilst t the time, a factor most easily measured, is eliminated.

With the culture of yeast growing in wort at 20° it was found that 10^6 yeast cells per c.c. caused a fermentation of 0·620 cm. per hour. In another experiment with growing yeast cells it was found that when the yeast crop was $5{\cdot}2 \times 10^6$ cells per c.c. the fermentation was 14·4 cm. That is

$$F = 0{\cdot}620 \times 10^{-6}, \quad N = 5{\cdot}2 \times 10^6, \quad S = 14{\cdot}4.$$

It follows therefore that

$$0{\cdot}434\,K = 0{\cdot}434\,FN/S = 0{\cdot}097$$

a result in agreement with the previous experiments.

Method 4.

The fourth method involves measurements of the rate of fermentation but has an advantage over method 2, as it allows measurements to be taken in any part of the reaction even where the yeast growth is retarded and nevertheless gives true values of K the constant of unrestricted growth.

The method is as follows: two experiments are made, the medium in each case being seeded with small seedings in a known ratio. If the seedings are small enough the time-fermentation curves are identical except that the one reaction is a definite time behind the other. This time-difference is the time for the one seeding to grow to the other, and from the two values K is easily determined.

If R is the ratio of the two seedings and t_1 and t_2 the times at which the two fermentations reach some definite stage in the reaction, then

$$0{\cdot}434\,K = \frac{1}{t_2 - t_1} \log R.$$

This method was tested in the following manner. Two flasks of wort were seeded with actively growing yeast, one 319 times the amount of the other. 75 c.c. of the wort with the greater seeding were placed in the tube of the apparatus and the manometer connected. Appreciable fermentation

started in 15 hours and several readings were taken at definite times. The first solution was then replaced by the second which in the meantime had been resting in the thermostat. On the next day the times were taken when the manometer showed the same changes in pressure as in the previous experiment. The various differences of time were in good agreement and the value of 0·434 K calculated from the average came to 0·106.

TABLE III.

t_1 = time at which the first reaction gives the reading M on the manometer scale.
t_2 = time on the next day when the second reaction reaches the same point.
Temp. = 20°. R = 319.

M	t_1	t_2	$t_2 - t_1$
9·4	9·20 a.m.	9·0 a.m.	23 hrs. 40 mins.
10·6	9·50	9·32	23 ,, 42 ,,
11·55	10·10	9·52	23 ,, 42 ,,
12·6	10·35	10·18	23 ,, 43 ,,
13·6	10·55	10·38	23 ,, 43 ,,
		Average	23 hrs. 42 mins. = 23·7 hrs.

$$0{\cdot}434\,K = \frac{\log 319}{23{\cdot}7} = 0{\cdot}106. \quad T = 2{\cdot}85 \text{ hrs.}$$

A comparison of the values of K determined by the different methods shows good agreement.

TABLE IV.

Method	1	gives	0·434 K = 0·102
,,	2	,,	0·434 K = 0·101
,,	3	,,	0·434 K = 0·097
,,	4	,,	0·434 K = 0·106

It is evident that the rate of growth of the yeast during this period is regular and can be measured accurately by yeast counting or by fermentation. It is of interest to note that the yeast crop at any time is composed mainly of the last few generations of yeast and any dying off of the old yeast cells would hardly affect the value of K. Further if the yeast crop were composed of cells of different activity correct values of K would be obtained by method 2 if the average value of the fermentative power remains constant during the time of measurement, for equation 6 a involves only ratios of S and s.

The equations and experiments cover only about 2 per cent. of the reaction corresponding to a yeast crop of 10 million cells per c.c., which is about 8 per cent. of the final crop. Growth after this time is measurably retarded, probably mainly by the carbon dioxide which is known to have a considerable retarding influence on yeast growth.

An accurate knowledge of the yeast crop and the fermentation during this early period is however given by these experiments and may be put in the following form.

TABLE V.

Temp. = 20°.
$F = 1{\cdot}2 \times 10^{-14}$ grms. per sec. $= 4{\cdot}3 \times 10^{-11}$ grms. per hour. $0{\cdot}434\,K = 0{\cdot}100$.

Hrs.	Cells per c.c.	G. per c.c. fermented
—	1,000	$1{\cdot}8 \times 10^{-7}$
10	10,000	$1{\cdot}8 \times 10^{-6}$
20	100,000	$1{\cdot}8 \times 10^{-5}$
30	1,000,000	$1{\cdot}8 \times 10^{-4}$
40	10,000,000	$1{\cdot}8 \times 10^{-3}$

These equations and methods of estimating the constant of growth can be applied to other micro-organisms. Methods of counting may not always be suitable, but method 4 is probably of general application. The rate of growth of bacteria which produce acid, for instance, could be determined by following the reaction by titration or by electrical conductivity and methods for other growths could be devised without much difficulty.

One of the principal uses of K is to determine the time between infection and the beginning of the chemical action brought about by the organism. The factors which influence K are the factors which determine this time and in extreme cases say whether a liquid is susceptible to the growth of the micro-organism or not.

The value of K may not remain constant over the period of growth in which one is specially interested but a row of constants is not the aim of the investigation and a consistent though variable K may lead to more important results than a constant one.

Micro-organisms grow not only in liquids but also on solids and in the liquid film covering solids and it is of importance to know whether these equations and methods are applicable to determine rates of growth in such cases.

Some information on the subject was obtained by measuring the rate of growth of this culture of yeast in solid wort-gelatin (10 g. gelatin per 100 c.c. wort). The sterile solid medium was melted and seeded with about 100,000 yeast cells per c.c. 75 c.c. were poured into a bottle which was filled with pieces of glass tubing, the whole being previously sterilised. There still remained about 80 c.c. air space. The melted gelatin was cooled by rotating the bottle under a jet of cold water so that the gelatin as it solidified became distributed over the tubing.

The bottle was then connected to the manometer and the apparatus

exhausted. Measurements of the rate of fermentation were then taken and the constant of growth calculated as in Table II

TABLE VI.

Temp. = 15°	t	$S+s$	$0{\cdot}434\,K$	
	−20	(0·1)	—	
	0	2·05 (S)	—	
	4	3·8	0·067	
	5	4·4	0·066	
	6	5·15	0·067	
	9·1	8·25	0·066	
	9·4	8·65	0·067	
	10·6	10·2	0·065	
	10·8	10·4	0·065	
		Average	0·066	$T=4{\cdot}6$ hrs.

The rate of growth of a yeast colony developing in wort-gelatin from a single cell therefore follows the logarithmic law. The experiments show that retarding influences do not come into play up to the time the colony consists of 200 yeast cells and probably the colony would grow regularly to a much larger size. It is of interest to note that diffusion would play no controlling part in determining the rate of fermentation until the colony consists of several million yeast cells. Slator and Sand [1910] have made calculations of the size of a yeast cell which would just ferment entirely the whole of the sugar diffusing to it in a stationary liquid. At 30° the radius is calculated to be 8×10^{-3} cm. The volume of such a yeast cell is 8 million times as great as one of radius 4×10^{-4} cm. Diffusion under these conditions would not be a limiting factor in the fermentation by a yeast colony until the colony consists of 8 million cells.

The rate of growth of this culture of yeast in wort gelatin is appreciably higher than in wort itself, the rates being 0·066 and 0·050 respectively.

The investigation shows the possibility of measuring rates of growth when the organism is growing on a solid medium.

REFERENCES.

Slator (1906), *J. Chem. Soc.* **89**, 128.
—— (1908), *J. Soc. Chem. Ind.* **27**, 653.
—— and Sand (1910), *J. Chem. Soc.* **97**, 927.